

CLAIMS

1. A method of preparing particles for immunoassays, comprising:
 reacting particles comprising carboxylate groups with N-
 hydroxysuccinimide or N-hydroxysulfosuccinimide and with a carbodiimide
 coupling reagent to provide activated particles comprising succinimide ester
 groups;

contacting said activated particles with antibodies to provide
 sensitized particles comprising covalently bound antibodies and residual
 succinimide esters; and

treating said sensitized particles in an aqueous mixture with an
 amine compound of formula (I):



wherein $-\text{X}$ is selected from the group consisting of $-\text{NH}_2$,
 $-\text{OH}$, and $-\text{CO}_2\text{CH}_2\text{CH}_3$; and

R is selected from the group consisting of an alkyl group
 and an alkyl ether group;

wherein, when $-\text{X}$ is $-\text{NH}_2$ or $-\text{CO}_2\text{CH}_2\text{CH}_3$, R comprises
 from 1 to 20 carbon atoms; and when $-\text{X}$ is $-\text{OH}$, R comprises from 4
 to 20 carbon atoms.

2. The method of claim 1, wherein
 $-\text{X}$ is selected from the group consisting of $-\text{OH}$ and $-\text{NH}_2$; and
 R is an alkyl ether group comprising from 4 to 20 carbon atoms
 and from 1 to 9 oxygen atoms.

3. The method of claim 1, wherein the amine compound is selected
 from the group consisting of glycine ethyl ester; 2-(aminoethoxy)ethanol; 2,2'-
 (ethylenedioxy)bisethylamine; and 4,7,10-trioxa-1,3-tridecanediamine.

4. The method of claim 1, wherein the ratio of equivalents of amine
 compound to equivalents of carboxylate groups is at least 50.

5. The method of claim 1, wherein the ratio of equivalents of amine compound to equivalents of carboxylate groups is at least 100.

6. The method of claim 1, wherein the ratio of equivalents of amine compound to equivalents of carboxylate groups is at least 200.

5 7. The method of claim 1, wherein the aqueous mixture has a pH of at least 7.0.

8. The method of claim 1, wherein the particles covalently bind less than 0.35 milligrams per square meter of non-specific protein when contacted with serum.

10 9. The method of claim 1, wherein the particles covalently bind less than 0.30 milligrams per square meter of non-specific protein when contacted with serum.

15 10. The method of claim 1, wherein the particles covalently bind less than 0.20 milligrams per square meter of non-specific protein when contacted with serum.

11. The method of claim 1, wherein the particles covalently bind less than 0.10 milligrams per square meter of non-specific protein when contacted with serum.

20 12. The method of claim 1, wherein the particles covalently bind less than 0.05 milligrams per square meter of non-specific protein when contacted with serum.

13. The method of claim 1, wherein the particles physically adsorb less than 3 milligrams per square meter of non-specific protein when contacted with serum.

25 14. The method of claim 1, wherein the particles physically adsorb less than 2 milligrams per square meter of non-specific protein when contacted with serum.

15. The method of claim 1, wherein the particles physically adsorb less than 1 milligram per square meter of non-specific protein when contacted with serum.

16. A sensitized particle for use in immunoassays, comprising:
a particle comprising a surface;
at least one antibody bound to the surface through a covalent bond; and
the reaction product of a succinimide ester and an amine compound of formula (I) on the surface;



wherein -X is selected from the group consisting of $-\text{NH}_2$, $-\text{OH}$, and $-\text{CO}_2\text{CH}_2\text{CH}_3$; and

R is selected from the group consisting of an alkyl group and an alkyl ether group;

wherein, when -X is $-\text{NH}_2$ or $-\text{CO}_2\text{CH}_2\text{CH}_3$, R comprises from 1 to 20 carbon atoms; and when -X is $-\text{OH}$, R comprises from 4 to 20 carbon atoms.

17. The sensitized particle of claim 16, wherein
-X is selected from the group consisting of $-\text{OH}$ and $-\text{NH}_2$; and
R is an alkyl ether group comprising from 4 to 20 carbon atoms and from 1 to 9 oxygen atoms.

18. The sensitized particle of claim 16, wherein the amine compound is selected from the group consisting of glycine ethyl ester; 2-(aminoethoxy)ethanol; 2,2'-(ethylenedioxy)bisethylamine; and 4,7,10-trioxo-1,3-tridecanediamine.

19. The sensitized particle of claim 16, further comprising BSA on the surface.

20. The sensitized particle of claim 16, wherein the particle comprising a surface is selected from the group consisting of gold particles, ceramic particles, and polymer particles.

21. The sensitized particle of claim 16, wherein the particles covalently bind less than 0.35 milligrams per square meter of non-specific protein when contacted with serum.

22. The sensitized particle of claim 16, wherein the particles covalently bind less than 0.30 milligrams per square meter of non-specific protein when contacted with serum.

23. The sensitized particle of claim 16, wherein the particles covalently bind less than 0.20 milligrams per square meter of non-specific protein when contacted with serum.

24. The sensitized particle of claim 16, wherein the particles covalently bind less than 0.10 milligrams per square meter of non-specific protein when contacted with serum.

25. The sensitized particle of claim 16, wherein the particles covalently bind less than 0.05 milligrams per square meter of non-specific protein when contacted with serum.

26. The sensitized particle of claim 16, wherein the particles physically adsorb less than 3 milligrams per square meter of non-specific protein when contacted with serum.

27. The sensitized particle of claim 16, wherein the particles physically adsorb less than 2 milligrams per square meter of non-specific protein when contacted with serum.

28. The sensitized particle of claim 16, wherein the particles physically adsorb less than 1 milligram per square meter of non-specific protein when contacted with serum.

29. A particle for use in immunoassays, comprising:
a polymer particle comprising a surface;
at least one antibody bound to the surface through a covalent bond;

5 BSA on the surface; and
the reaction product of a succinimide ester and an amine compound on the surface;

wherein the amine compound is selected from the group consisting of glycine ethyl ester; 2-(aminoethoxy)ethanol; 2,2'-(ethylenedioxy)bisethylamine; and 4,7,10-trioxa-1,3-tridecanediamine;

wherein the particles covalently bind less than 0.35 milligrams per square meter of non-specific protein when contacted with serum; and

wherein the particles physically adsorb less than 2 milligrams per square meter of non-specific protein when contacted with serum.

30. A reagent, comprising:
a plurality of particles;
each of said particles comprising a surface;
an antibody bound to the surface through a covalent bond; and
the reaction product of a succinimide ester and an amine compound of formula (I) on the surface;



wherein -X is selected from the group consisting of $-\text{NH}_2$, $-\text{OH}$, and $-\text{CO}_2\text{CH}_2\text{CH}_3$; and

R is selected from the group consisting of an alkyl group and an alkyl ether group;

wherein, when -X is $-\text{NH}_2$ or $-\text{CO}_2\text{CH}_2\text{CH}_3$, R comprises from 1 to 20 carbon atoms; and when -X is $-\text{OH}$, R comprises from 4 to 20 carbon atoms.

31. The reagent of claim 30, wherein

—X is selected from the group consisting of —OH and —NH₂; and

R is an alkyl ether group comprising from 4 to 20 carbon atoms and from 1 to 9 oxygen atoms.

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32. The reagent of claim 30, wherein the amine compound is

selected from the group consisting of glycine ethyl ester; 2-

(aminoethoxy)ethanol; 2,2'-(ethylenedioxy)bisethylamine; and 4,7,10-trioxa-1,3-tridecanediamine.

33. An assay method for determining an antigen, comprising:

combining a sample suspected of containing said antigen with the reagent of claim 30,

the reagent comprising the antibody of said antigen, and

the reagent capable of forming a detectable complex with said antigen;

and

determining the presence or amount of said detectable complex as a measure of said antigen in said sample.

34. A test kit, comprising the reagent of claim 30.